云 南 植 物 研 究 2009, 31 (6): 477～485
Acta Botanica Yunnanica DOI: 10.3724 SP.J.1143.2009.09165

盐适应过程中香根草内源游离态、结合态、束缚态多胺含量的变化

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摘要: 研究了不同浓度 NaCl 胁迫下, 香根草 (*Vetiveria zizanioides*) 根、叶中的游离态、结合态、束缚态多胺 (PAs) [包括腐胺 (Put), 尸胺 (Cad), 亚精胺 (Spd) 和精胺 (Spm)] 含量的变化。在中度盐胁迫 (100, 200 mmol L^{-1} NaCl) 9 天时, 香根草基本能够正常生长, 但在重度盐胁迫 (300 mmol L^{-1} NaCl) 下, 其生长受到严重抑制。在上述 3 个不同浓度的 NaCl 胁迫下, 香根草根、叶中游离态 Put, Cad, Spd, Spm 和总的游离态 PAs 含量明显下降, 在高盐浓度下下降幅更大; 结合态 Put, Cad, Spd, Spm 和总的结合态 PAs 含量显著上升, 但在重度盐胁迫下升幅较小或与对照相当; 束缚态 Put, Cad 和总的束缚态 PAs 含量均减少, 而束缚态 Spd 和 Spm 含量在叶中是下降的, 在根中则增加, 且在中度盐胁迫下更明显。对根和叶片而言, 除游离态 (Spd + Spm) Put 比值在重度盐胁迫下较对照显著下降外, 其它游离态、结合态、束缚态和总的 (Spd + Spm) Put 比值在不同盐胁迫下均上升, 在中度盐胁迫下更明显。这表明, 维持多胺总量的稳态和较高的 (Spd + Spm) Put 比值是香根草适应中度盐胁迫的一个重要机制。

关键词: 多胺; 盐胁迫; (Spd + Spm) Put 比值; 香根草
中图分类号: Q 945 **文献标识码**: A **文章编号**: 0253-2700 (2009) 06-477-09

Changes in Free, Conjugated and Bound Polyamine Content in Salt Adaptation of Vetiver Grass (*Vetiveria zizanioides*, Poaceae)

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Abstract: Polyamines (PAs) have been suggested to play roles in plant salt stress adaptation. In this study, alteration of free, conjugated and bound PAs [putrescine (Put), cadaverine (Cad), spermidine (Spd) and spermine (Spm)] in roots and leaves of vetiver grass (*Vetiveria zizanioides*) seedlings in response to salt stress were investigated. Under moderate salt stresses (100 and 200 mmol L^{-1} NaCl) for 9 days, vetiver grass grew at similar vigor compared with seedlings under normal growth conditions. However, growth was severely arrested when plants were treated with severe salt stress (300 mmol L^{-1} NaCl). Under three different concentrations of NaCl stress mentioned above, the contents of free Put, Cad, Spd, Spm and total free PA substantially decreased in roots and leaves, and more severe loss of free PAs was observed under higher NaCl concentration. Conjugated Put, Cad, Spd, Spm, and total conjugated PAs remarkably increased, and the extent of the increase after 300 mmol L^{-1} NaCl treatment was smaller than those after 100 or 200 mmol L^{-1} NaCl treatments. Bound Put, Cad and total bound PAs decreased in both roots and leaves under salt stress, moreover, bound Spd and Spm decreased in leaves while increased in roots in response to salt treatments, and the more obvious rise was displayed under moderate salt stresses. With the exception of the significant decreases of free (Spd + Spm) Put ratio in roots and leaves after 300 mmol

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L^{-1} NaCl treatment, other ratios of free, conjugated, bound and the total (Spd + Spm) Put increased in roots and leaves of vetiver grass seedlings after different salt treatments, and especially under moderate salt stress. These results indicated that maintaining homeostasis of total PAs content and high (Spd + Spm) Put ratios could be an adaptation mechanism in vetiver grass to moderate saline environment.

Key words: Polyamines; Salt stress; (Spd + Spm) Put ratio; Vetiver grass (*Vetiveria zizanioides*)

Salinity is one of the most deleterious abiotic stresses, which adversely influences plant growth, development and crop productivity in the world (Liu *et al.*, 2008). Salinity can harm the plant by its osmotic and ionic effects (Zhu, 2003), which disrupts the integrity of cellular membranes, uptake of essential nutrients, function of photosynthetic apparatus and many other physiological and biochemical processes (Demetriou *et al.*, 2007). On the other hand, plants have evolved mechanisms to cope with salt stress, one of which is to synthesize some organic substances, such as polyamines (PAs), glycine betaine, proline, and sugars (Bartels and Sunkar, 2005).

PAs are small organic cations with aliphatic nitrogen and widely observed in different organisms ranging from bacteria to plants and animals. In plants, PAs are involved in various physiological and developmental events such as senescence and stress responses (Alcázar *et al.*, 2006). The common PAs are diamine putrescine (Put), cadaverine (Cad), triamine spermidine (Spd), and tetramine spermine (Spm) (Bouchereau *et al.*, 1999). Plant PAs occur as free, conjugated and bound forms, the most common conjugated Put, Spd, Spm are those that are covalently linked to hydroxycinnamic acids catalyzed by Put, Spd and Spm hydroxycinnamoyl transferases, i.e. PHT, SHT and SpmHT, respectively. The conversion of free PAs to bound PAs is mostly catalyzed by Transglutaminase (TGase, EC.2.3.2.13) (Martin-Tanguy, 2001). Plant PAs contents were shown to be altered in response to salt stress (Vasuki and Astrid, 2004). Application of exogenous PAs was also reported to improve plant salt tolerance (Liu *et al.*, 2006). Plant PAs are supposed to act as free radical scavengers, cellular membrane protectors and maintaining cellular ionic balance during salt stress response (Jiménez-Bremont *et al.*, 2007). High accumulation of free Spd and Spm, or bound PAs were beneficial for plant to alleviate the salt injury, while accumulation of Put had adverse effect on plant salt tolerance (Zhao *et al.*, 2003).

Vetiver grass (*Vetiveria zizanioides*) is a graminaceous plant native to tropical and subtropical areas. Its unique morphological, physiological and ecological characteristics render this plant species capable of growing in harsh environmental and soil conditions (e.g. drought, hot, barren, acid, salt and alkali, and heavy metal, etc.). This plant species has been used for soil and water conservation, rehabilitation of mines, contaminated soil and saline land, as well as wastewater treatment. Besides active studies on its biological traits, current research has been focused on using this plant species for conservation of soil and water, phytoremediation of heavy metals and other pollutants-contaminated fields (Pang *et al.*, 2003; Lai and Chen, 2004). Klomjek and Nitisoravut (2005) explored the feasibility of using this plant to remove pollutants from saline wastewater. Our former work has showed that, accumulation of inorganic and organic osmolytes in vetiver grass seedlings under moderate saline stress (less than 200 mmol L^{-1} NaCl), can play a good role in the osmotic adjustment and salt adaptation (Zhou and Yu, 2009). However, the role of PAs in salt tolerance response of vetiver grass has not been reported. In this study, we investigated the changes in free, conjugated and bound PAs in roots and leaves of vetiver grass under different salt stresses, and the possible function of PAs in salt adaptation of vetiver grass was also discussed.

1 Material and methods

1.1 Plant Materials and Growth Conditions

Vetiver grass seedlings were obtained from hypaethral experimental garden located in the city of Nanjing, P.R. China. Six-month-old vetiver grasses with multiple stems were divided into single seedlings, which were clipped to keep leaf length with about 0.3 m and root length with about 0.15 m. These seedlings

were fixed in the sheet of foam board, its roots were dipped into 1 2 Hoagland solution in plastic square boxes, and were cultured at 28 ± 2℃ day 22 ± 2℃ night under light irradiation of 500 mmol (m^2 s) (16 h per day) in the growth chamber.

1.2 Salt Treatments

When seedlings resumed growing, they were subjected to the treatments with following solutions: 1 2 Hoagland solution (Control), 1 2 Hoagland solution + 100 mmol L^{-1} NaCl, 1 2 Hoagland solution + 200 mmol L^{-1} NaCl, and 1 2 Hoagland solution + 300 mmol L^{-1} NaCl. All the above solutions were replaced by fresh solution in every 2 days and their pH were adjusted to 6.0. After treatment for 9 days, the vetiver grass seedlings were sampled and tested.

1.3 Determination of Plant Height Growth Rate

Plant height was directly measured using a centimeter ruler at 0 and 9 days, respectively, and the plant height growth rate was calculated using the following formula:

Plant height growth rate = $(H_9 - H_0)$ days

H_0 and H_9 represented the height of seedlings at 0 and 9 days, respectively. The plant height growth rate was expressed as means ± SE (cm d) of 6 seedlings.

1.4 Extraction and Analysis of PAs

Free, conjugated and bound PAs were extracted and quantified according to Kotzabasis *et al*. (1993) with minor modifications. 0.5 g (fr wt) of root or leaf samples were homogenized in 5 ml of 5% (v v) perchloric acid (PCA) water solution and incubated at 4℃ for 1 h. The homogenate was centrifuged at 10 000 g for 30 min at 4℃ and the supernatant and pellet were collected separately. The supernatant was used to determine the free and conjugated PAs, and the pellet was used to measure the PCA-insoluble bound PAs as follows:

In order to extract the PCA-soluble conjugated PAs, 2 ml of the supernatant was mixed with 2 ml of 12 mmol L^{-1} HCl and heated at 110℃ for 18 h in flame-sealed glass ampules. After acid hydrolysis, HCl was evaporated from the tubes by further heating at 80℃ and the residue was resuspended in 1 ml of 5% (v v) PCA. This solution was used as a source of PCA-soluble conjugated PAs and free PAs.

For extracting PCA-insoluble bound PAs, the pellet was rinsed 4 times with 5% (v v) PCA to remove any trace of soluble PAs and then dissolved by vigorous vortexing in 2 ml of 1 mmol L^{-1} NaOH. The mixture was centrifuged at 10 000 g for 30 min and the supernatant, including the bound PAs, was hydrolysed under the same conditions as PCA-soluble conjugated PAs.

Free PAs extracted from non-hydrolyzed supernatant, free plus conjugated PAs extracted from hydrolyzed supernatant and bound PAs extracted from pellet were derived with benzoyl chloride as previously described (DiTomaso *et al*., 1989). Briefly, 0.5 ml of the above supernatant solutions was mixed with 1 ml of 2 mol L^{-1} NaOH. After the addition of 10 μl benzoyl chloride, vortexing for 20 s, and incubation for 20 min at 37℃, 2 ml of ether was added. After centrifugation (1 500 g for 5 min), 1ml of the ether phase was collected, evaporated to a dry state, and redissolved in 100 μl of methanol (HPLC grade). Benzoyl-polyamines (10 μl) were analyzed using a Waters HPLC System (USA) equipped with an isocratic pump and a reverse phase C_{18} column (Nova-pak, 150×3.9 mm, particle size 4 μm). Methanol acetonitrile H_2O (48∶2∶50) (v v v) was used as an isocratic eluting solvent at a flow rate of 0.8 ml min^{-1}. Polyamine peaks were detected by a Perkin-Elmer LC-95 absorbance detector at 254 nm. Conjugated polyamine contents were calculated by subtracting the free polyamines from the acid-soluble polyamine contents. The fresh weight (fr wt) of plant sample was transformed into dry weight (dry wt) through the sample water content, and the PAs content was expressed as nmol g dry wt.

1.5 Data Analysis

Each value was expressed as means ± SE of three independent experiments. The means of the different treatments with three separate experiments were used to calculate *t*-values for statistical analysis. Different letters show significant difference ($P < 0.05$). All data of the experiments were analyzed by using SPSS 13.0.

2 Results

2.1 Effect of NaCl Stress on Height Growth Rate of Vetiver Grass Seedlings

Treatments with 100, 200 or 300 mmol L^{-1} NaCl for 9 days resulted in significant decrease of plant height growth rate compared with the control (Fig. 1a). However, the plant appearance under moderate salt stress (100 and 200 mmol L^{-1} NaCl) was as normal as the control plant did. Severe growth arrest and salt injury were observed on seedlings treated with severe salt stress (300 mmol L^{-1} NaCl) (Fig. 1b). This result indicated that vetiver grass has moderate salt tolerance capacity.

2.2 Effect of NaCl Stress on Free PAs Contents

Put and Spd were the most abundant free PAs in roots and leaves of vetiver grass seedlings. When compared with the control, the contents of free Put, Cad, Spd, Spm and total free PAs decreased in both roots and leaves after 100, 200 or 300 mmol L^{-1} NaCl treatments for 9 days. Treatment with higher concentrations of NaCl resulted in more severe losses of free PAs

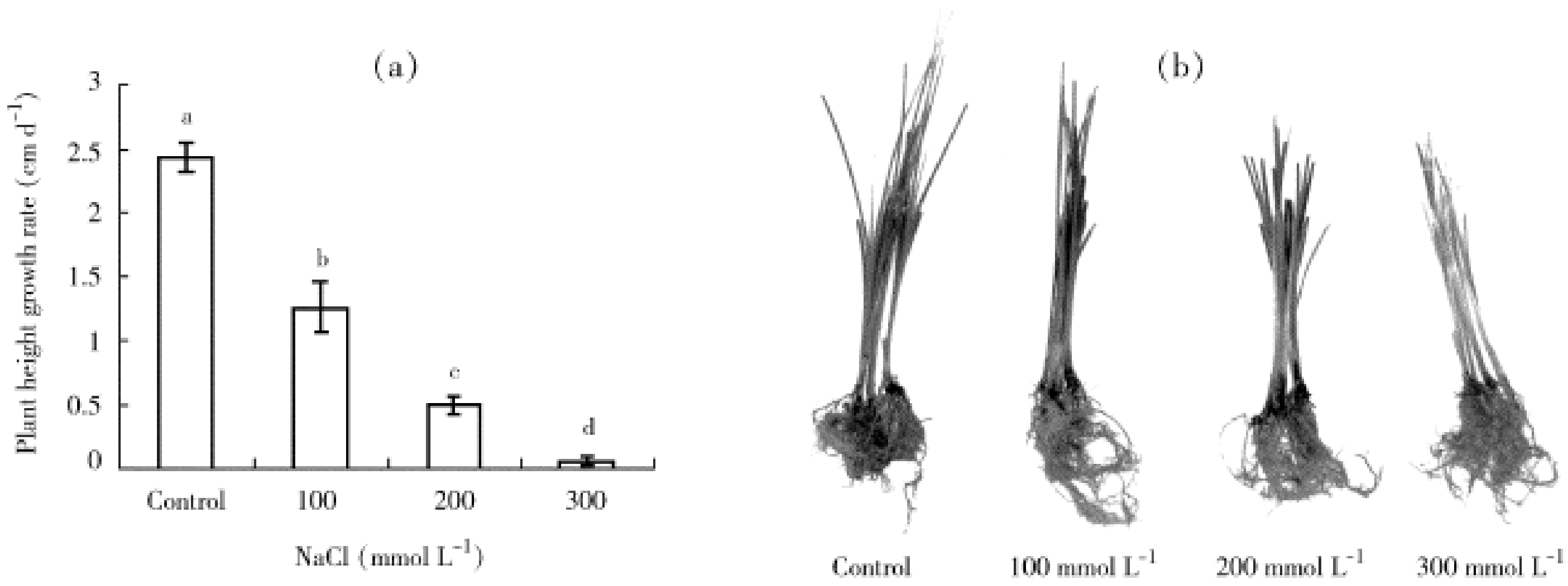

Fig. 1 Effects of NaCl with different concentrations on the plant height growth rate (a) and plant growth appearance (b) of vetiver grass seedlings stressed for 9 days. Plant height growth rate was measured and expressed as means ± SE ($n = 6$). Different letters show significant difference ($P < 0.05$)

(Fig. 2: a-e). The ratios of free (Spd + Spm) Put increased in roots and leaves after 100 or 200 mmol L^{-1} NaCl treatments, whereas the significant decreases were observed in roots and leaves after 300 mmol L^{-1} NaCl treatment (Fig. 2f).

2.3 Effect of NaCl Stress on Conjugated PAs Contents

Conjugated Put, Cad, Spd, Spm and total conjugated PAs significantly increased in roots and leaves of vetiver grass seedlings after treated with 100 or 200 mmol L^{-1} NaCl for 9 days. The increase after 200 mmol L^{-1} NaCl treatment was greater than that with 100 mmol L^{-1} NaCl treatment. Although contents of conjugated PAs were still higher in seedlings with 300 mmol L^{-1} NaCl treatment than in control seedlings, the extent of the increase after 300 mmol L^{-1} NaCl treatment was smaller than those after 100 or 200 mmol L^{-1} NaCl treatments (Fig. 3: a-e). The changes in ratio of conjugated (Spd + Spm) Put in roots and leaves showed the same trend as above-described. It was noticeable that the ratios of conjugated (Spd + Spm) Put after 100 or 200 mmol L^{-1} NaCl treatments were similar and higher than that with 300 mmol L^{-1} NaCl treatment (Fig. 3f).

2.4 Effect of NaCl Stress on Levels of Bound PAs

Bound Put was found to be the most abundant bound PAs in roots, which accounted for about 90% of the total bound PAs. When compared with the control, the contents of bound Put, Cad and total bound PAs decreased in both leaves and roots after salt stress treatments. However, bound Spd and Spm increased in roots and decreased in leaves (Fig. 4: a-g). In roots, the bound (Spd + Spm) Put ratios significantly increased after salt stresses, and 200 mmol L^{-1} NaCl treatment resulted in highest increase of the ratios (Fig. 4h). The bound (Spd + Spm) Put ratios in leaves increased after 100 or 200 mmol L^{-1} NaCl treatments, and declined after 300 mmol L^{-1} NaCl treatment, but the alteration on the ratios was not significant when compared with the control (Fig. 4i).

3 Discussion

Many previous studies have suggested that alteration in polyamine forms and levels plays an important role in plant abiotic stress response (Alcázar *et al.*, 2006; Bouchereau *et al.*, 1999). However, there are some contradictory reports on changes in PAs content under stresses (Basu *et al.*, 1988; Yang *et al.*, 2007). This discrepancy might be resulted from plant species, type and development stage of tissues employed, intensity and duration of stress treatments, and types of stresses for experiments (Botella *et al.*, 2000). Thus, the precise role of PAs metabolism in plant stress response still remains elusive (Alcázar *et al.*, 2006).

In our present study, for the first time, we simultaneously focused on the changes in contents of endoge-

nous free, conjugated and bound PAs and their total in vetivar grass seedlings under salt stress with different NaCl intensity. Some remarkable changes in contents of different forms of PAs were observed in roots and leaves of vetiver grass seedlings after 100, 200 or 300 mmol L^{-1} NaCl stress for 9 days. The contents of free Put, Cad, Spd and Spm and their total in roots and leaves of vetiver grass seedlings significantly decreased in salt-stressed seedlings than in control seedlings (Fig. 2: a-e). Bound Put, Cad, Spd, Spm and their total also

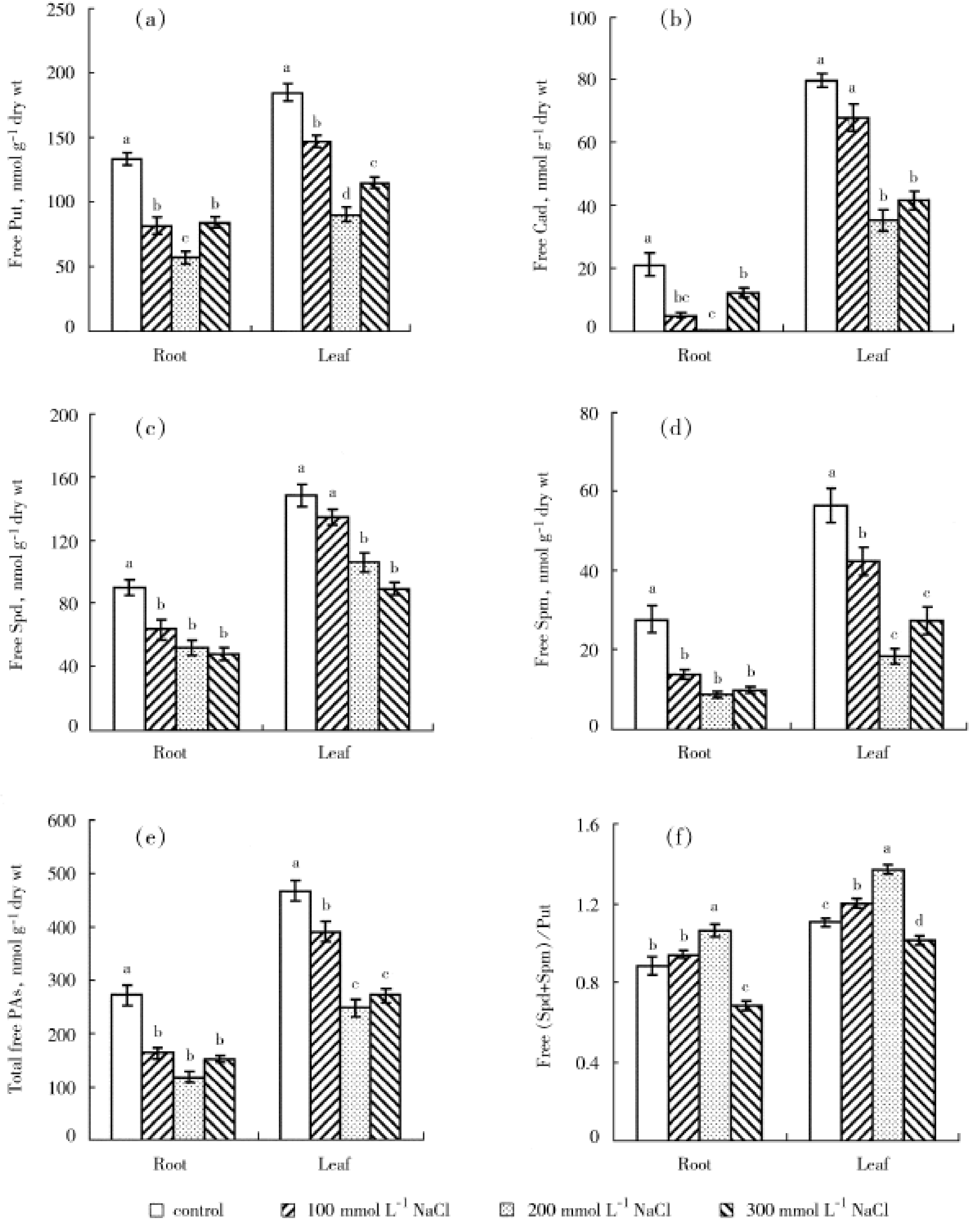

Fig. 2　Changes in content of free Put (a), Cad (b), Spd (c), Spm (d), their total (e) and free (Spd + Spm) Put ratio (f) in roots and leaves of vetiver grass seedlings under 100, 200, 300 mmol L^{-1} NaCl for 9 days, respectively. Polyamine levels are expressed as means ± SE (n = 3) of three independent experiments. Different letters show significant difference ($P < 0.05$)

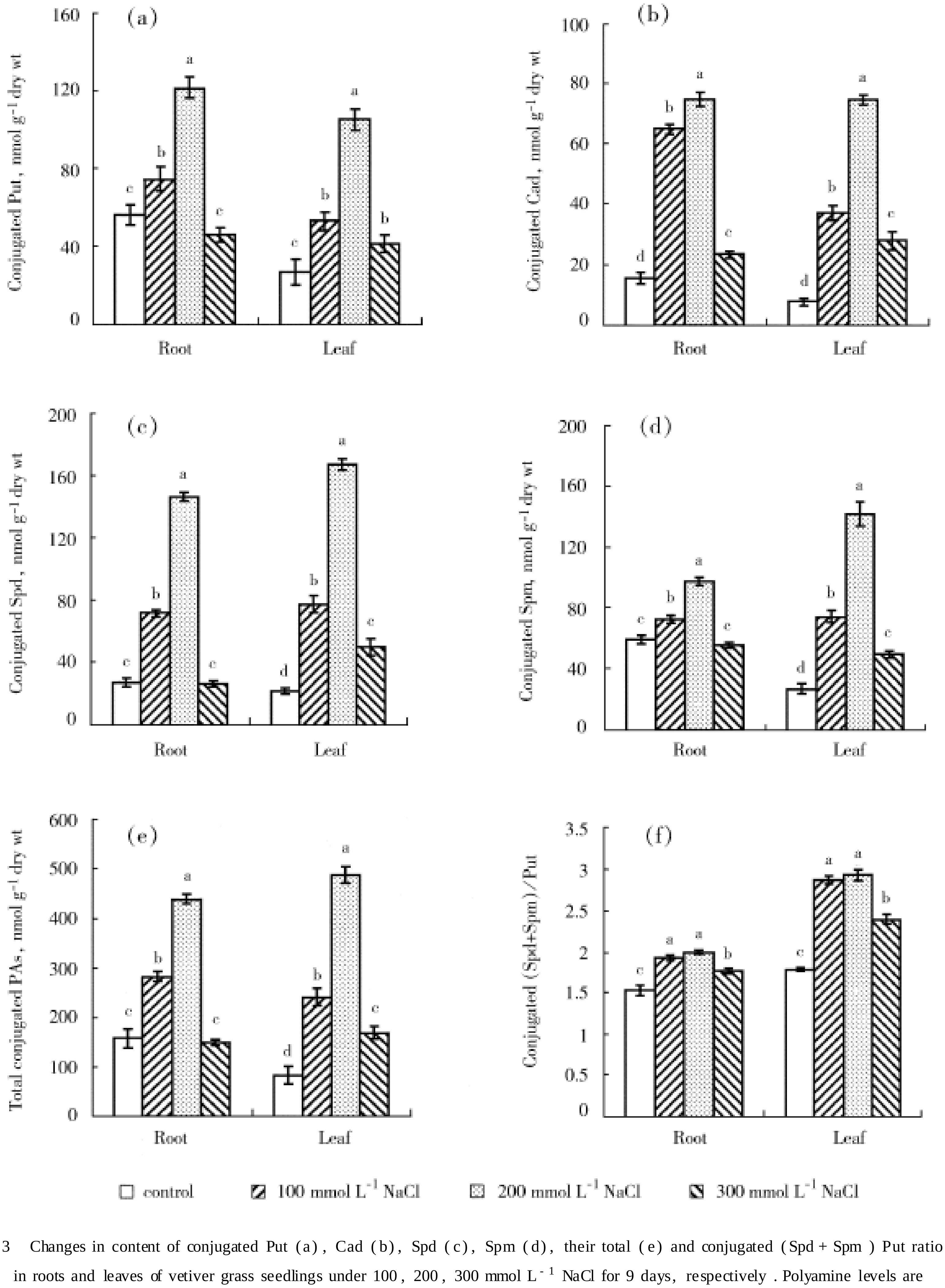

Fig.3 Changes in content of conjugated Put (a), Cad (b), Spd (c), Spm (d), their total (e) and conjugated (Spd + Spm) Put ratio (f) in roots and leaves of vetiver grass seedlings under 100, 200, 300 mmol L^{-1} NaCl for 9 days, respectively. Polyamine levels are expressed as means ± SE (n = 3) of three independent experiments. Different letters show significant difference ($P < 0.05$)

showed similar changes as the above-described with the exception that bound Spd and Spm in roots was increased (Fig.4: a-g). The decrease of free PAs was accompanied with decline of total bound PAs in both roots and leaves of vetiver grass seedlings under 100, 200 and 300 mmol L^{-1} NaCl stress (Fig.2; Fig.4: f, g). The contents of total PAs including free, conjugated and bound forms could be maintained relatively homeostatic in roots and leaves of vetiver grass seedling under moderate salt stresses (100 and 200 mmol L^{-1}

NaCl), which could be attributed to the increase of conjugated PAs for compensating the decrease of free and bound ones. However, under severe stress condition (300 mmol L^{-1} NaCl), the total PAs in roots and leaves significantly declined when compared with the control (Fig. 5a).

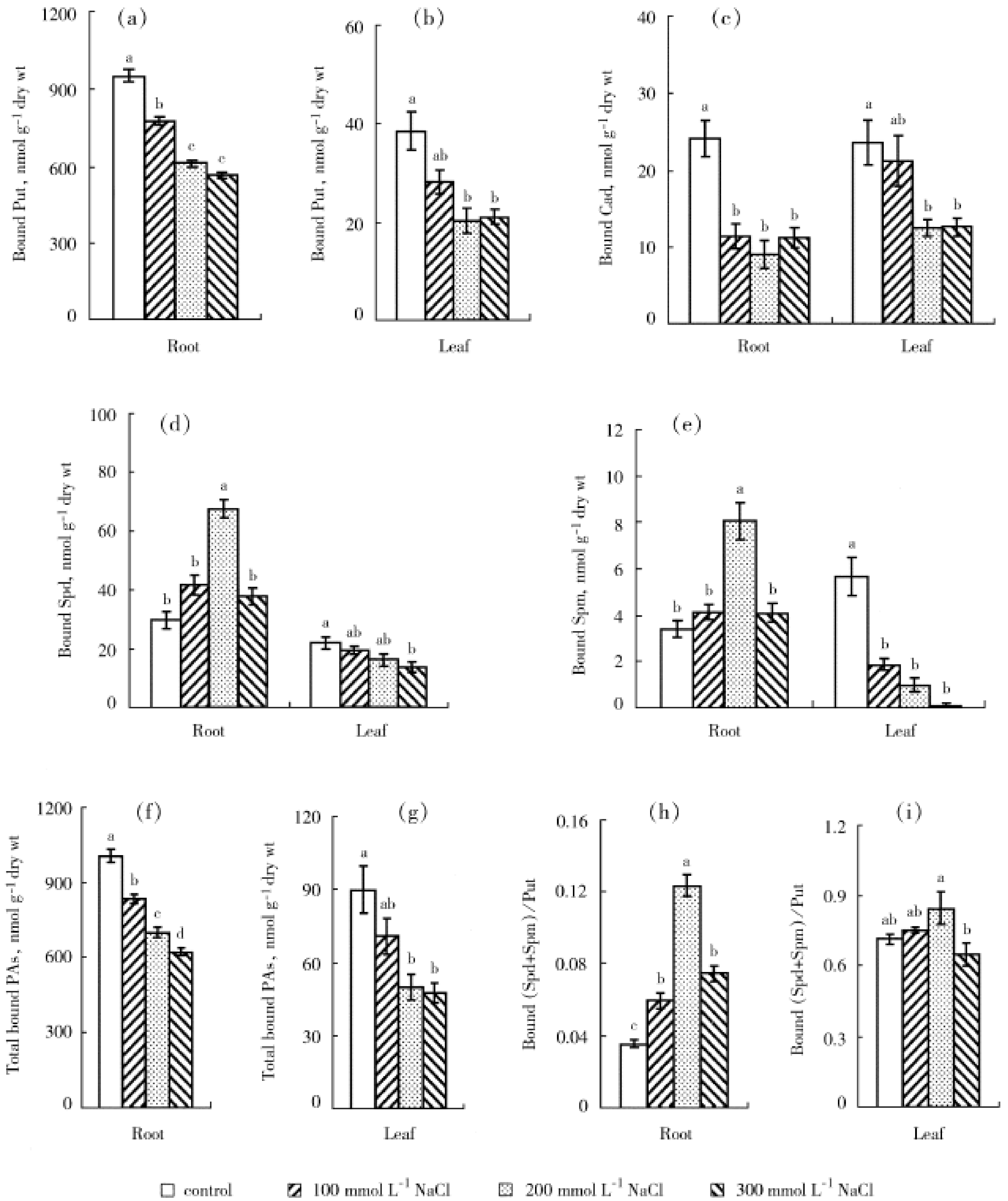

Fig. 4 Changes in content of bound Put (a for root, b for leaf), Cad (c), Spd (d), Spm (e), their total (f for root, g for leaf) and bound (Spd + Spm) Put ratio (h for root, i for leaf) in vetiver grass seedlings under 100, 200, 300 mmol L^{-1} NaCl for 9 days, respectively. Polyamine levels are expressed as means ± SE ($n = 3$) of three independent experiments. Different letters show significant difference ($P < 0.05$)

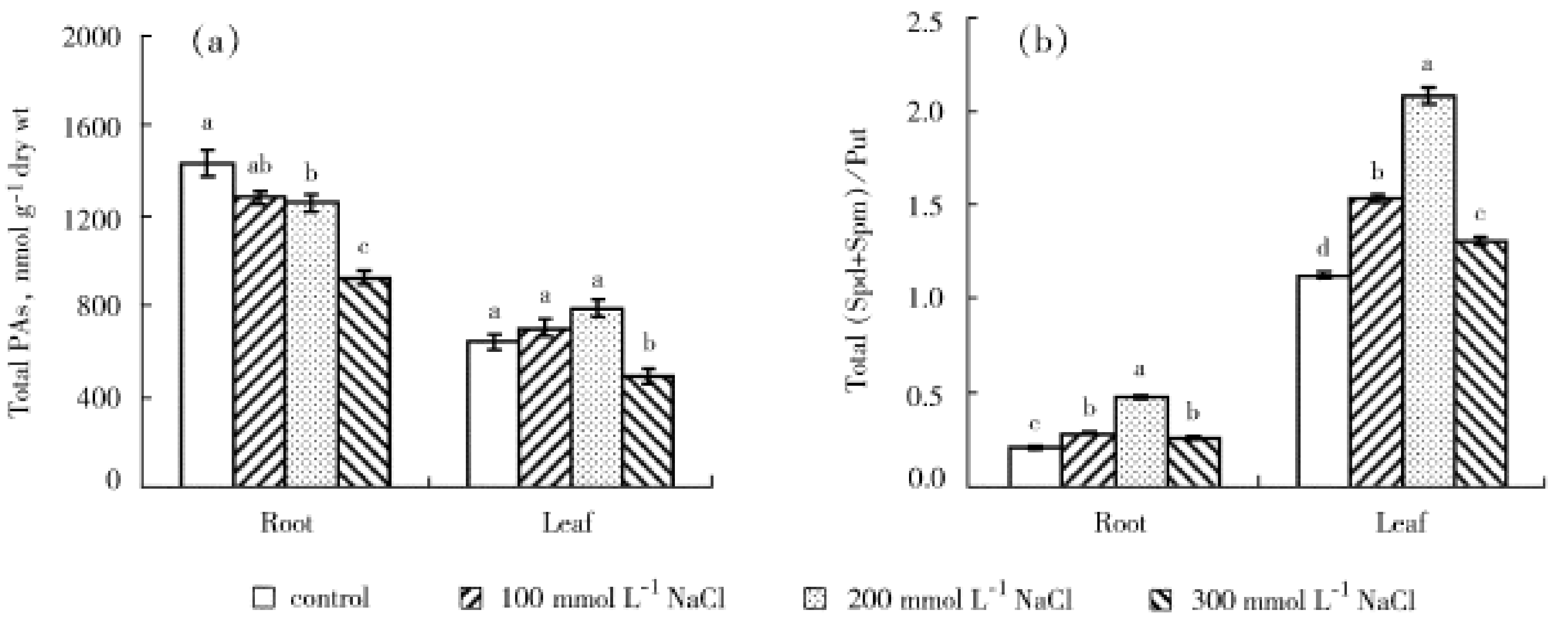

Fig . 5 Changes in total PAs content (a) and total (Spd + Spm) Put ratio (b) in roots and leaves of vetiver grass seedlings under 100, 200, 300 mmol L^{-1} NaCl for 9 days, respectively . The values are expressed as means ± SE (n = 3) of three independent experiments . Different letters show significant difference (*P* < 0.05)

Although there is little study on the function of conjugated PAs in plant response to salt stress, it has been shown that the conjugated PAs are associated with plant resistance to other stresses . For example, the conjugated PAs were important in enhancing ability of plant to tolerate diseases (Walters, 2003) . Piqueras *et al*. (2002) found that high concentration of conjugated diamines could be correlated with the establishment of normal growth rate in hyperhydric carnation plants . The conversion of free PAs to conjugated PAs enhanced the chilling-tolerance of potato (Mauricio *et al*., 1999) . Rodríguez-Kessler *et al*. (2008) found that conjugated PAs be related to the mounting of defense mechanisms of the plant against the infection of the pathogen . The above mentioned may attribute to their functions, for example, they could be the preferred substrates for amine oxidases, stabilize cell membrane and regulate of the free PA titers and or in detoxicating phenolic compounds (Martin-Tanguy, 2001) . In our work, it is very noticeable that, the contents of conjugated Put, Cad, Spd, Spm and their total increased significantly in roots and leaves of vetiver grass seedlings under moderate NaCl stresses (100 or 200 mmol L^{-1} NaCl), while the increase was not significant after severe salt stress (300 mmol L^{-1} NaCl) when compared with the control (Fig.3: a-e) . The decrease of free PAs in vetiver grass seedlings under moderate NaCl stress is very likely resulted from the conversion or biosynthesis of conjugated ones . Our data indicate that, moderate and severe salt stress would cause distinct alternations on PA forms and levels, leading to either adaptation and or salt injury in vetiver grass seedlings, and which can maintain the homeostasis of total PAs during no-deadly and longer term saline stress . This is also consistent with the different plant growth appearance of vetiver grass seedlings under moderate and severe salt stress (Fig.1: a, b) .

Under salt stress conditions, salt-tolerant plants often have higher (Spd + Spm) Put ratio than salt-sensitive plants, suggesting a protective role of Spd and Spm in plants against saline environment (Sannazzaro *et al*., 2007) . The increase in the (Spd + Spm) Put ratio might be one of the crucial factors for plant stress tolerance, in partly, for reducing free radicals and alleviating lipid peroxidation, as free-radical scavengers or through the induction of the activities for several antioxidant enzymes such as superoxide dismutase, catalase, and ascorbate peroxidase (Wen *et al*., 2008; Wang *et al*., 2007) . In this study, a increase of free (Spd + Spm) Put ratios was also observed in salt-stressed vetiver grass seedlings, although free PAs in roots and leaves of vetiver grass decreased when compared with the control (Fig.2: a-f) . Higher conjugated and bound (Spd + Spm) Put ratios were also found in vetiver grass seedlings after salt stress treatments (Fig.3f; Fig.4: h, i) . Consistently, the ratios of to-

tal (Spd + Spm) Put were also higher in salt-stressed seedlings than the control plants, especially for the moderate salt-stressed ones (Fig.5b). Thus, maintenance of high (Spd + Spm) Put ratio may be related to the salt adaptation of vetiver grass.

In conclusion, our results indicate that, maintaining the homeostasis of total PAs by increasing the conjugated PAs to compensate the decrease of free and bound ones, and keeping high (Spd + Spm) Put ratios may jointly contribute to the adaptation of vetiver grass to moderate saline environment.

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